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BIO-ASSAY INVESTIGATIONS, NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION, BUFFALO, NEW YORK

Croswell Henderson, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1956

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BIO-ASSAY INVESTIGATIONS

FOR

INTERNATIONAL JOINT COMMISSION

NATIONAL ANILINE DIVISION

ALLIED CHEMICAL AND DYE CORPORATION

BUFFALO, NEW YORK

1956

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

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Cincinnati, Ohio

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Bio-Assay Investigations

National Aniline Division

Allied Chemical and Dye Corporation

Buffalo, New York

Prepared by

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Introduction

Purpose

In January 1956, field studies were initiated on a cooperative basis to determine the possible effects of the wastes from major Buffalo River industries on the eastern end of Lake Erie and the Niagara River. Buffalo River industries cooperating in this project were the National Aniline Division-Allied Chemical and Dye Corporation, Donner-Hanna Coke Corporation, and the Socony Mobil Oil Company.

This report covers an investigation of the toxicity to fish of intake waters and major effluents from the National Aniline Division-Allied Chemical and Dye Corporation. Chemical and dye wastes contain some chemical compounds

which are known to be toxic to aquatic life in low concentrations. Some of these chemicals when mixed with or under the influence of other non-toxic components of the effluent or receiving water may exert an entirely different toxicity from that of the pure compounds. Bio-assays were made to evaluate directly the toxicity of these chemically complex wastes.

Personnel Participating

National Aniline Division

Dr. K. W. Hess, Coordinator of Pollution Research

C. J. Carney, Chemist

J. A. Gouck, Chemist

Public Health Service

Hayse H. Black, Industrial Wastes Consultant

Croswell Henderson, Aquatic Biologist

Dr. C. M. Tarzwell, Chief, Aquatic Biology Unit

H. A. Anderson, Public Health Engineer

M. W. Ruszaj, Chemist

Organization of Study

National Aniline Division personnel collected 24 hour composite samples of their intake water and effluents and determined the volume of effluents discharged. The Public Health Service conducted bio-assays in laboratory space provided by National Aniline and made phenol and cyanide determinations in the IJC Unit laboratory. Phenol and cyanide determinations were also made on some effluent samples by the National Aniline Pollution Research Laboratory.

General Information

Production Operations

National Aniline Division is one of the largest producers of dyes, dye intermediates, and synthetic organic chemicals, including surface active agents, textile assistants, plastic intermediates, and pharmaceuticals in the United States. Batch type operations are used in the production of many of the numerous products of the Buffalo plant. The products being manufactured at any one time vary greatly according to market demand and stock on hand.

Water Supply

Buffalo city water is used for drinking, sanitary purposes, and as process water. The major portion of the plant water supply is used for process cooling and is obtained from the Buffalo River through a shore intake located about 100 feet upstream from the National Aniline Plant "A" Sewer outfall and downstream from plant "B&C", and "E" sewers. During periods of low river flow, there is some recycling of the plant effluents.

Process Wastes

Wastes are discharged into the Buffalo River through three separate outfalls designated as plants "A", "B&C", and "E" sewers. All of these sewer outfalls are on the North bank of the Buffalo River. Plant "A" outfall is located 400 feet downstream from South Park Bridge, plant "B&C" outfall immediately south of the bridge, and plant "E" outfall about 1000 feet upstream from the bridge.

Each of the above sewers receives wastes from numerous and varied production processes. The wastes in each may be expected to vary significantly at times due to changes in quantity or type of products being manufactured.

Waste Control Measures

1. For the past 20 years the company has supported and continues to support

a program of research on the effects of and methods for treating its wastes and its full scale application to reducing existing pollution and minimizing or avoiding pollution by new processes.

2. For this purpose an especially equipped laboratory has been provided and manned with specially trained personnel for the entire period.

3. During recent years a pilot plant has been provided and operated for investigating the chemical and biochemical treatment of objectionable wastes which might result from expansions of production in the future.

4. This program is integrated into the entire organization.

5. New Processes, equipment installations and replacements are not operated until approved for wastes control by the Pollution Research Unit.

6. Two units for neutralization of waste acids with lime have been constructed and are operated when necessary to maintain a pH of at least 6 in the Buffalo River.

7. Some wastes are segregated and discharged to the Buffalo Sewage System, with written approval by the Buffalo Sewer Authority, for which fees are paid.

8. Wastes containing arsenic are placed in containers and disposed of by burial 100 miles at sea.

9. Certain wastes, including sulfides and thiosulfates, are destroyed by chemical treatment in especially designed equipment.

10. Some oily wastes are separated and used as fuel.

11. Some oils, tars, and various other kinds of combustible wastes are separated and burned in an especially designed incinerator.

12. Non-combustible solids and sludges, including metallic compounds, are separated and deposited in dumps or sludge ponds for eventual sale or disposal in a manner to avoid pollution.

13. Aluminum chloride wastes are sent to the Buffalo Sewage Treatment Plant where they are used to advantage as a sludge conditioner.

14. Other wastes are recovered, refined and used in plant production processes.

Survey Methods

Flow Measurements

Waste flow in the sewers was determined by National Aniline personnel. Flow in the Plant "E" Sewer was determined by a water level recorder which measured the height of the liquid in a sewer. This device had previously been calibrated by Pitot tube measurements.

Flows in plants "A" and "B&C" Sewers were estimated from the volume of intake water. City intake water was measured by positive displacement meters and river water intake was measured by orifice meters.

Following are the measured daily flows during a one week period. The average values were used in all bio-assay computations:

		1000 gal./day	
Date	Plant "A"	Plants "B&C"	Plant "E"
1-19	11,349	7,231	1,753
1-20	11,265	7,341	1,680
1-21	11,225	6,113	1,770
1-24	11,151	6,529	2,090
1-25	11,386	6,898	1,918
Average mgd	11.30	6.82	1.84
cfs	17.4	10.5	2.8

Sampling

Twenty-four hour composite effluent samples were collected by means of automatic samplers. Samples of Plant "A" Sewer were collected at the outfall. Plant "B&C" samples were collected through a riser located 15 feet upstream from the sewer outfall. Plant "E" samples were collected from the influent to the waste treatment pilot plant. This influent is pumped from a point in the sewer approximately 1000' above the sewer mouth.

Chemical Methods

All chemical determinations were made according to procedures in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes", 10th edition, published by the American Public Health Association.

Bio-assay Methods

Bio-assays were made, essentially, by the method recommended by the toxicity subcommittee of the Federation of Sewage and Industrial Wastes Associations (Sewage and Industrial Wastes, Vol. 23, No. 11, 1380, Nov. 1951). This method consists of preparing various concentrations of effluent in a selected dilution water, adding the test fish and observing their reactions over a definite time period. A logarithmic series of numbers is generally most convenient for preparing different test concentrations.

As the effluents were of unknown toxicity, exploratory or small scale tests were made to determine the approximate toxic range. Test solutions were prepared over a wide range of concentration (e.g. 100, 1, and 0.1 percent effluent). Two fish were added to 2 liters of each concentration in 6-1/2 inch diameter, 1-gallon wide mouth glass bottles. Observations for relatively short time periods indicated test concentrations necessary for the full scale experiments,

In the full scale tests, 10 fish were generally used for each test concentration. Five fish were added to 10 liter duplicate samples in 10 inch diameter, 5-gallon widemouth glass bottles. The intermediate concentrations tested were dependent upon information obtained from the exploratory tests. For example, if fish were killed in concentrations above 10 percent and not affected in concentrations of 1 percent, intermediates were set up within this range (e.g., 10, 5.6, 3.2, 1.8, and 1.0 percent concentration of effluent).

The dilution water used was raw Lake Erie water obtained at the Buffalo, N. Y., water plant. This water was hauled into the laboratory, allowed to come to room temperature, and aerated vigorously for at least one hour to bring it to equilibrium with atmospheric gases. Characteristics of this water at time of use were as follows: Dissolved oxygen 7.6-8.2 ppm; pH 8.0-8.2;

Total alkalinity ($CaCO_3$) 94-100 ppm; Total acidity ($CaCO_3$) 0-1 ppm; Versenate hardness ($CaCO_3$) 120-135 ppm.

The test fish used were fathead minnows (Pimephales promelas) of a fairly uniform size, ranging in length from 2 to 2-1/2 inches and in weight from 1 to 1-1/2 grams. These fish were obtained in uniform lots from the Newtown, Ohio, Fish Hatchery and acclimated to laboratory conditions. This species is of intermediate tolerance to chemicals comparable to bass, sunfish, perch, and other warm water species, and will tolerate fairly low oxygen conditions (1-2 ppm).

Another species, the emerald shiner (Notropis atherinoides) obtained locally from a Buffalo bait dealer was used in some experiments in order to obtain a comparison of the toxicity to fatheads and this locally abundant species.

The bio-assays were made at ordinary laboratory temperatures which were within the range from 22 to 25° C. and would compare favorably with maximum summer water temperatures in this area.

The tests were designed so that no oxygenation or aeration was generally needed. Absorption of atmospheric oxygen by the exposed water surface was normally adequate for fish requirements during the test period. However, in some of the bio-assays, high oxygen demand effluents caused oxygen depletion. When necessary, oxygen was maintained by bubbling pure oxygen through the test solution by means of a suitable arrangement of valves and small tubing. The rate (60-180 bubbles per minute) was adjusted to maintain adequate oxygen for fish survival with minimum agitation to prevent the loss of volatile materials.

Physical and chemical determinations (temperature, dissolved oxygen, pH, alkalinity, and hardness) were made on each concentration both initially, after

fish mortality, or at the completion of the test. This was done primarily to maintain control of oxygen conditions and to differentiate between fish mortality due to oxygen deficiency or acidity and other toxic properties.

Fish reactions were observed over a 96 hour period. From the mortality in the different concentrations, 24, 48, and 96 hour TL_m (Median Tolerance Limit) values were obtained. The Median Tolerance Limit is the concentration of effluent in dilution water that kills just 50 percent of the test fish. These values were obtained by straight line graphical interpolation from percent survival of fish and log concentrations bracketing the 50 percent point.

Effluent samples were normally brought into the laboratory in the morning immediately following collection and exploratory tests set up. Observations by late afternoon indicated the toxic range and full scale tests were then set up.

As some of the effluents were highly acid and at pH values which would be rapidly fatal to fish, similar bio-assays were set up with portions of sample that had been neutralized with lime to pH 7.

Bio-assays were also made on mixtures of "A", "B&C", and "E" effluents, mixed in proportion to the volumes released into the Buffalo River.

Results

Physical and Chemical Characteristics

A general description and some of the chemical characteristics of the plants "A", "B&C", and "E" effluents and the river intake waters are shown in Table 1.

All of these effluents were highly colored, with the intensity or shade varying somewhat in the different composite samples. The "A" effluent was generally from green to blue, the "B&C" effluent from orange to red, and the "E" effluent from pink to dirty-orange. A mixture of these effluents gave a dark

brown color, similar to, but of higher intensity than, that of Buffalo River intake water.

Odors were variable in intensity but some samples of "A" and "E" effluents smelled of very strong solvents (chlorobenzene, etc.). Little or no odor was noticeable in the "B&C" effluent. All effluents were relatively clear, containing only a small amount of suspended matter, oil, or other extraneous material. Some samples, especially the "E" effluent, formed precipitates when added to the dilution water.

Dissolved oxygen values were high in most "A" and "E" effluent samples and low values were reflected only when river intake values were also low. Evidently organic materials in these effluents are very slowly oxidizable. "B&C" samples, however, were either very low in or free of dissolved oxygen.

With the exception of two samples of "A", all effluents were highly acid with pH values ranging from 2 to 4 and acidity values from 100 to 1200 ppm. The "E" effluent was normally the most acid, followed by "B&C" and then "A". When neutralized with lime to pH 7, medium to heavy precipitates formed in the "B&C" and "E", but little or none in the "A" effluents. A color change from blue to green was apparent in "A" samples when neutralized.

Phenols were highest in "B&C" effluent samples ranging upward to about 2 ppm with other effluents containing generally less than 0.5 ppm. Cyanides were at very low values in all effluents.

TABLE 1 - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
Plant "A" Effluent	1-26	Dark purplish-blue. Faint solvent. Slight turbidity.	6.0	3.0	0	120	300	820	943	<5
24-Hr. Composite	2-15	Dark greenish-blue. Burnt rubber. Slight turbidity.	8.0	2.8	0	325	175	248	146	<5
	2-16	Dark blue. Burnt rubber. No turbidity.	6.8	3.8	0	100	150	113	136	<5
	2-23	Dark bluish-green. Strong burnt rubber. No turbidity.	9.0	3.3	0	150	145	-	-	-
	3-14	Greenish-blue. Faint solvent. Slight turbidity.	6.8	5.8	24	30	130	474	535	<5
	3-21	Bright green. Sweetish solvent. No turbidity.	6.6	3.0	0	155	185	137	134	<5
	6-21	Dark green . Faint solvent. No turbidity.	0.0	7.8	144	10	135	-	-	-

TABLE 1 (Cont'd) - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
Same effluents as above. Neutralized with lime	2-15	Changed color from blue to green. No precipitate.	8.0	7.2	54	16	305	-	-	-
	2-23	Slight precipitate.	8.4	7.4	58	20	330	-	-	-
Plant "B&C" Effluent 24-Hr. Composite	1-18	Dark reddish-orange. Faint solvent. Slight turbidity.	0.0	2.6	0	380	740	1026	1180	<5
	1-19	Dark red. Faint solvent. Slight turbidity.	-	2.4	-	-	-	-	-	-
	2-14	Dark reddish-orange. No odor. No turbidity.	2.0	2.9	0	660	200	1983	1836	<5
	2-21	Dark orange-red. No odor. No turbidity.	2.0	3.2	0	350	280	2091	2039	<5
	3-13	Dark orange-red. Faint burnt rubber. No turbidity.	0.0	2.9	0	810	480	1014	1071	12
	3-21	Dark orange-red. No odor. Slight turbidity.	0.0	2.5	0	600	300	-	-	-

TABLE 1 (Cont'd) - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
	6-20	Dark orange-red. Sweetish solvent. No turbidity.	0.0	2.6	0	420	520	-	210	<5
Same effluent as above.	1-19	Fairly heavy brown precipitate.	-	7.0	-	-		-	-	-
Neutralized with lime.	2-14	Dark brown precipitate.	-	7.0	-	-	-	-	-	-
	2-21	Small amount of brown precipitate.	-	7.0	-	-	-	-	-	-
	3-13	Heavy dark brown precipitate.	-	6.7	44	18	1050	-	-	-
Plant "E" Effluent 24-Hr. Composite	1-24	Light orange. Strong solvent (chlorobenzene). No turbidity.	-	1.9	0	770	240	864	896	<5
	2-15	Light pink. Faint solvent(sweet). No turbidity.	9.4	3.0	0	280	150	774	739	<5
	2-17	Light dirty-orange. Faint solvent(sweet). Slight turbidity.	7.0	2.7	0	480	125	-	166	<5
	2-21	Light tan. Faint solvent(sweet). Slight turbidity.	10.0	2.5	0	1020	135	461	422	<5

TABLE 1 (Cont'd) - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
	3-13	Dirty bluish-gray. Strong solvent (butyl aldehyde). Slight turbidity.	8.8	2.6	0	960	135	182	179	20
	3-20	Pink. Solvent (sweet). No turbidity.	4.4	2.3	0	1200	180	168	181	<5
	3-21	Pink. Strong solvent (sweet). No turbidity.	10.4	2.5	0	680	185	-	-	-
	6-20	Light dirty-orange. Solvent. No turbidity.	0.0	2.6	0	245	210	-	182	<5
Same effluents as above. Neutralized with lime	1-24	Light colored precipitate.	-	7.0	-	-	-	-	-	-
	2-17	Fairly heavy light colored precipitate.	6.8	7.4	30	4	650	-	-	-
	2-21	Heavy light colored flocculent precipitate.	7.6	7.3	90	8	1150	-	-	-
	3-13	Dark brown flocculent precipitate-settles to clear solution.	-	7.4	90	0	1130	-	-	-

TABLE 1 (Cont'd) - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
Mixed "A", "B&C", and "E" effluents. In proportion to volume released.	A -3-14 B&C-3-13 E -3-13	Dark purplish-brown. Sweetish solvent. Slight turbidity.	4.0	2.6	0	320	230	-	-	-
	A, B&C, and E-3-21	Dark brown color. Solvent odor. Slight precipitate.	4.4	2.6	0	400	280	-	-	-
	A -6-21 B&C-6-20 E -6-20	Dark red-orange. Sweetish solvent. Slight turbidity.	-	5.5	24	100	325	-	-	-
River Intake water 8-Hr. Composite	1-18	Brown. Faint oil & sewage. Slight turbidity.	8.0	6.7	72	30	216	651	980	<5
	2-14	Light grayish-tan. Faint sewage. Slight turbidity.	11.0	7.3	80	6	135	92	79	<5
	2-16	Light grayish-tan. Faint sewage. Slight turbidity.	7.6	7.6	64	4	110	52	29	<5
	2-21	Light grayish-tan. Gasoline & sewage. Slight turbidity.	9.0	7.5	66	6	125	70	73	<5

TABLE 1 (Cont'd) - PHYSICAL AND CHEMICAL DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppb 4AA	Phenols ppb Gibbs	Cyanide ppb
	3-13	Light grayish-tan. Faint gasoline. Slight turbidity.	10.0	7.5	72	4	115	11	10	5
	3-21	Grayish-tan. Faint gasoline. Slight turbidity.	10.8	7.2	86	12	155	-	-	-
	6-21	Dark brown. Faint solvent. Slight turbidity.	0.4	6.8	72	30	250	-	-	-

Toxicity to Fish

A summary of bio-assay results showing the direct toxicity of the effluents to fish is shown in Table 2. The information on which these results are based, including concentrations tested, time for fish mortality, percent of mortality, and bio-assay control data are shown in the Appendix.

Median tolerance limit (TL_m) values were obtained for 24, 48, and 96 hour periods. The 96 hour TL_m was used to compute the dilution ratio, which is the ratio of a unit volume of effluent to dilution water which produces a 50 percent mortality of the test fish. This ratio times the effluent flow gives the dilution volume or the total flow or volume of receiving water required to reduce the toxicity of the effluent to where a 50 percent mortality of the test fish is produced. The following formula may be used for directly computing this dilution volume:

$$\frac{100 - TL_m}{TL_m} \times \text{Effluent Flow} = \text{Dilution Volume}$$

The dilution volumes represent conditions which would cause direct injury to fish upon short time exposure and are a direct comparative measure of toxicity. Liberal application factors must be applied to these results for complete protection of aquatic life.

All samples of "A", "B&C", and "E" effluents were toxic to fish. "A" and "E" were about equally toxic and "B&C" considerably less. The toxicity was variable with 96 hour TL_m values ranging from 4.2 to 15 percent for the "A"

effluent, 13.5 to 28 percent for the "B&C", and 4.0 to 24 percent for the "E" effluent. Intake water samples were relatively non-toxic, a significant mortality of fish occuring only in the June (low flow) intake sample. The difference in 24 and 48 hour TL_m values for the "A" effluent was significantly greater than for the others, which may reflect some chronic or accumulative toxicity.

Neutralization with lime did not significantly reduce the toxicity of the "A" or "E" effluents but considerably reduced that of the "B&C" effluent. In some of the samples, the addition of dilution water to the raw effluent raised pH values to "safe" levels and yet a rapid mortality of fish occurred. Neutralization would not be expected to reduce toxicity very much in these situations.

Some difficulty was encountered in maintaining adequate oxygen in the high concentrations of neutralized "B&C" effluent. Whether fish loss was due to oxygen depletion or toxicity could not be clearly distinguished. This difficulty was also encountered in a few samples of the "A" effluent. In most samples of "A" and "E", oxygen was adequate for fish survival without maintaining it artificially.

The effluents were more toxic to shiners (Notropis atherinoides), a locally important forage species, than to fathead minnows.

Mixing the "A", "B&C", and "E" effluents did not significantly alter the toxicity in two of three samples. In the others the toxicity was reduced by one-half.

TABLE 2 - SUMMARY OF BIOASSAY DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Effluent Flow cfs	TLm (Median Tolerance Limit) (Per cent Concentration) 24 hr.	48 hr.	96 hr.	Dilution Ratio	Dilution Volume cfs
Plant "A" Effluent 24-hr. Composite	1-26	17.4	10	4.2	4.2	1:22.8	396
	2-15	"	20	16	15	1:5.7	98
	Neutralized		42	24	21		
	2-16	"	20	13.5	13.5	1:6.4	112
	2-23	"	24	11	11	1:8.1	141
	Neutralized		24	-	-		
	3-14	"	10	7.5	7.5	1:12.3	214
	3-21	"	16	7	6.2	1:15.1	264
	Shiners		7	4.5	4.5		
	6-21	"	7.5	6.2	6.2	1:15.1	257(1)
Average		17.4			9.1		212

(1) Intake toxicity subtracted.

TABLE 2 (Cont'd) - SUMMARY OF BIOASSAY DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Effluent Flow cfs	TLm (Median Tolerance Limit) (Per cent Concentration) 24 hr.	48 hr.	96 hr.	Dilution Ratio	Dilution Volume cfs
Plant "B&C" Effluent 24 hr. Composite	1-18	10.5	28	28	28	1:2.6	27
	1-19	"	24	24	24	1:3.2	33
	Neutralized		75	-	-		
	2-14	"	21	19	18	1:4.6	48
	Neutralized		65	42	-		
	2-21	"	22	22	16	1:5.3	55
	Neutralized		>56	47	47		
	3-13	"	13.5	13.5	13.5	1:6.4	67
	Neutralized		>56	39	35		
	3-21	"	13.5	13.5	13.5	1:6.4	67
	Shiners		8	6.2	6.2		
	6-20	"	24	24	24	1:3.2	28(1)
Average		10.5			19.6		46

(1) Intake toxicity subtracted.

TABLE 2 (Cont'd) - SUMMARY OF BIOASSAY DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Effluent Flow cfs	TLm (Median Tolerance Limit) (Per cent Concentration) 24 hr.	48 hr.	96 hr.	Dilution Ratio	Dilution Volume cfs
Plant "B" Effluent 24 hr. Composite	1-24	2.8	6.2	4.2	4.0	1:24	67
	Neutralized		7.5	7.5	5.6		
	2-15	"	24	24	24	1:3.2	9
	2-17	"	7.5	6.2	4.2	1:22.8	64
	Neutralized		8	7	4.2		
	2-21	"	7.5	7.5	7	1:13.3	37
	Neutralized		22	16	16		
	3-13	"	7.5	7.5	7.5	1:12.3	35
	Neutralized		>18	>18	>18		
	3-20	"	7.5	5.6	5.6	1:16.9	47
	3-21	"	4.5	4.2	4.2	1:22.8	64
	Shiners		2.4	2.4	2.4		
	6-20	"	20	20	20	1:4	10(1)
Average		2.8			9.6		42
Mixed "A", "B&C", and "E" Effluents	A -3-14 B&C-3-13 E -3-13 3-21-56	30.7 "	9 13.5	9 13.5	9 13.5	1:10.1 1:6.4	310 197
In proportion to volume released	A -6-21 B&C-6-20 E -6-20	"	13.5	11	8.7	1:10.5	322

(1) Intake toxicity subtracted.

TABLE 2 (Cont'd) - SUMMARY OF BIOASSAY DATA

NATIONAL ANILINE EFFLUENTS

Source	Date 1956	Effluent Flow cfs	TLm (Median Tolerance Limit) (Per cent Concentration) 24 hr.	48 hr.	96 hr.	Dilution Ratio	Dilution Volume cfs
River Intake Water 8-hr.composite	1-18	30.7	100% surv.	90% surv.	70% surv.(2)		-
	2-14	"	100% surv.	100% surv.	100% surv.		-
	2-16	"	100% surv.	100% surv.	100% surv.		-
	2-21	"	90% surv.	90% surv.	90% surv.		-
	3-13	"	100% surv.	100% surv.	90% surv.		-
	3-21	"	100% surv.	100% surv.	100% surv.		-
	Shiners	"	100% surv.	100% surv.	80% surv.		
	6-21	30.7(3)	75	70	70	1:0.43	12

(1) Intake toxicity subtracted.

(2) Per cent survival of fish in undiluted sample.

(3) River water used by plant. Dilution volume was subtracted from that for plant effluents to determine that contributed by effluents.

Significance of Data

Characteristics which may Affect Aquatic Life

While bio-assays have shown plant "A", "B&C", and "E" effluents to be toxic to fish, insufficient information was available to indicate the major components responsible. The highest toxicity, however, was generally in effluents with a strong solvent odor. The chemically complex nature of these wastes and the numerous processes from which they are derived may make it quite difficult to determine sources of toxic materials. Bio-assays would be of considerable help in tracing this toxicity to process effluents.

Bio-assays conducted on phenol and ortho-cresol under experimental conditions similar to that of the effluents gave 96 hour TL_m values of 40 and 24 ppm, respectively. This would indicate that phenol was not toxic to fish at levels found in the effluents. Other phenolic compounds, especially some of the chlorophenols, have been reported toxic to fish in concentrations as low as 0.1 ppm.

While the quantity and nature of the solvents in these wastes are not known, many hydrocarbon solvents are toxic to fish. Bio-assays with some solvents gave 96 hour TL_m values for benzene, 56 ppm, for toluene, 51 ppm, and for xylene, 28 ppm.

Sub-lethal concentrations of phenols and benzene derivatives, such as chlorobenzene, are known to impart a taste to fish flesh.

Cyanide concentrations in these effluents were at levels which would not be expected to effect aquatic life.

The high acidity and corresponding low pH values of these effluents would have a detrimental effect on aquatic life, unless adequate buffering capacity was available in the receiving water or neutralization was affected before release. Normally, pH values below 5 may be expected to cause fish mortality. Even above lethal levels, decreasing the pH may have considerable effect on increasing the toxicity of certain materials, especially metal salts and sulfides. For example, larger amounts of metal ions such as copper, lead, zinc, iron, etc., are in solution at lower pH values. A decrease in pH of 1 unit may cause a 10 to 100 fold increase in the toxicity of some metals. Likewise, decreasing the pH releases hydrogen sulfide from sulfides and the toxicity may be greatly increased. Concentrations of H_2S as low as 1 ppm are known to have an effect on aquatic life. A reduction in pH may have an effect not only on materials released in these effluents but on those released by other industries and present in the receiving waters.

Color, apparently, has no direct effect on fish. No relationship could be established between color intensity and toxicity in any of the effluents. An indirect effect would be possible by a decrease in light penetration and consequently the productivity in receiving waters.

Oxygen depleting characteristics may have a definite effect on aquatic life unless adequate volumes of receiving water are available for dilution or assimilation. Dissolved oxygen concentrations should be maintained at 5 ppm or above in receiving waters for complete protection of aquatic life.

The release of large volumes of effluents at high temperatures which increase those in receiving waters may have an effect in increasing the toxicity of some materials, reducing dissolved oxygen, and a direct effect on some aquatic organisms.

Effect on Receiving Waters

All of these effluents were toxic to fish. The dilution volumes necessary to reduce this toxicity to 50 percent mortality of the test fish (Table 2) would obviously not provide for complete protection of aquatic life. Liberal application factors must be used, which are based on the following major considerations:

(1) The bio-assay procedure measures 50 percent mortality during a relatively short time period in non-renewed solutions. This must be related to no effect from continuous long time exposure.

(2) Test data apply directly to the species of fish used. While fathead minnows are of intermediate tolerance and compare favorably with many warm water "game" fishes, some locally important species of fish and fish food organisms may be more sensitive. Bio-assays conducted with a locally important forage fish, the emerald shiner (Notropis atherinoides), indicated this species was approximately twice as sensitive as fathead minnows.

(3) Some effluents may vary in toxicity. A few samples may give an indication of toxicity but maximum conditions may be missed. In many situations, maximum conditions of toxicity are the limiting factor as far as aquatic life is concerned.

(4) Some conditions which may generally reduce but in some cases increase the magnitude of the application factor are the loss of volatiles, hydrolysis, oxidation, precipitation, and changes in water quality characteristics by the entrance of other effluents.

Based on the best available information an application factor of at least 3 would be necessary to prevent fish kills and as much as 10 for complete protection and propagation of fish. It is evident that low seasonal flows in the Buffalo River would not provide this necessary dilution; however, only a small portion of the Niagara River flow would be necessary.

This contribution of toxicity to the Buffalo River adds to that present from other industries in the basin. While the toxicity from individual industrial effluents is substantially reduced in the basin, enough remains to present a threat to the Niagara River under abnormal conditions. Toxicity builds up in the Buffalo River during low flows and then may be rapidly flushed into the Niagara River. Additional dilution water in the Buffalo River during low flow periods would minimize this possibility. As long as large quantities of unknown toxic materials are entering the Buffalo River basin, it cannot be stated with certainty that threat to aquatic life in the Niagara River will be completely eliminated.

While oxygen depleting characteristics of these effluents were somewhat masked by high toxicity in the bio-assays, depletion of oxygen was observed in "B&C" and some "A" samples. As the volume of these effluents may exceed flows in the Buffalo River during low flows, oxygen depletion would no doubt occur in the river. This would not significantly affect the Niagara River but may have an effect in a border zone where the Buffalo River enters Lake Erie.

The possibility also exists that an off-flavor in fish flesh may be produced by small quantities of phenols, solvents, or other chemicals.

Conclusions

Bio-assays have shown that all samples of Plants "A", "B&C", and "E" effluents were toxic to fish. The toxic components were not identified. River intake samples were generally non-toxic or had only a slight degree of toxicity.

The "A" effluent was the most significant from the standpoint of toxicity contributed to the Buffalo River. The "A" and "E" effluents were equally toxic but the volume of "A" was much larger. The "B&C" effluent was considerably less toxic.

Neutralization with lime significantly reduced the toxicity of the "B&C" effluent but had only a slight or no effect on samples of "A" and "E" effluents. Most of the "A" and "E" raw samples remained toxic after dilution with Lake Erie water to concentrations with pH values which would be tolerated by the test fish.

Some oxygen depletion was apparent in concentrations of "B&C" effluent during the 96 hour test period. When this effluent was neutralized and oxygen maintained, very little toxicity remained. Most samples of "A" and "E" effluent, however, were toxic in concentrations that retained adequate oxygen for fish support.

These effluents are major contributors of toxicity to the Buffalo River and add substantially to that contributed by other industries in the basin. Seasonal low flows in the Buffalo River do not provide for adequate dilution of these wastes. Under normal flow conditions, however, little or no effect on aquatic life in the Niagara River would be expected.

The buildup of toxicity in the Buffalo River basin during low flows, with the possibility of a rapid release into the Niagara River under abnormal conditions does present some threat to aquatic life in the Niagara. Additional dilution water in the Buffalo River during low flows would minimize this possibility.

As long as large quantities of unknown toxic materials are entering the Buffalo River basin, the threat to aquatic life in the Niagara River cannot be completely eliminated. Bio-assays could be used to trace sources of toxic materials within a plant which would be of help in further reducing or eliminating the major toxic wastes.

APPENDIX TABLE A1 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOW
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant A	1-26-56	100										Effluent		6.0	3.0	0	120	300	Dark purple
Effluent																			Slight solvent odor
24-Hr. Composite		56	2	2	30 min.	30 min.	30 min.	0	0	0	0	Initial		-	4.6	-	-	-	Little turbidity
In Lab												30 Min.							Low pH
9:30 AM 1-26		32	5	10	1 hr.	1.5 hrs.	1.5 hrs.	0	0	0	0	Initial		7.2	6.2	36	18	185	
Fish Added												1.5 hrs.		7.0	6.4	40	12	175	
11:30 AM 1-26		18	10	20	3 hrs.	4 hrs.	5 hrs.	0	0	0	0	Initial	23	7.4	6.6	60	14	160	
												5 hrs.	23	6.8	6.8	64	16	155	
		10	10	20	6-20 hrs.	6-20 hrs.	24-40 hrs.	50	0	0	0	Initial		7.6	7.0	76	10	140	
												48 hrs.		0.2	7.1	88	8	155	Low D.O.
		5.6	10	20	24-46 hrs	24-46 hrs	24-46 hrs	100	0	0	0	Initial		7.4	7.4	84	6	135	
												48 hrs.		1.0	7.2	96	8	175	Possible low D.O.
		Control	10	20	-	-	-	100	100	100	100	Initial		7.4	8.2	94	2	125	
												96 hrs.		4.6	7.7	100	6	145	
		TLm (Median Tolerance Limit) - Per cent concentration						10	<4.2	<4.2	<4.2								
Plant A	2-15-56	100										Effluent		8.0	2.8	0	325	175	Dark greenish blue
Effluent																			Burnt rubber odor
24-Hr. Composite		56	2	2	30 min.	30 min.	30 min.	0	0	0	0	Initial		-	-	-	-	-	Little turbidity
In Lab																			
11:00 AM 2-15		32	10	20	30 min.	30 min.	30 min.	0	0	0	0	Initial	25	8.0	3.8	0	70	135	Low pH
Fish Added												30 min.	25	7.5	3.8	0	68	135	
3:00 PM 2-15		18	10	20	3-17 hrs.	27-42 hrs		60	40	30	30	Initial		8.0	6.0	32	30	135	
												96 Hrs.		5.0	7.4	36	10	130	
		10	10	20				100	100	100	100	Initial		8.0	6.4	64	18	130	
												96 Hrs.		4.0	7.5	84	6	130	
		Control	10	20				100	100	100	100	Initial		8.0	8.0	96	2	125	
												96 hrs.		4.2	7.7	100	2	130	
		TLm (Median Tolerance Limit) - Per cent concentration						20	16	15	15								
Plant A	2-15-56	100										Effluent		8.0	7.2	54	16	305	Color changed from blue to green
Same as above																			No precipitate
Neutralized		56	5	10	2-20 hrs.	2-20 hrs.	23 hrs.	0	0	0	0	Initial	25	8.0	7.6	70	8	225	
With lime												23 hrs.	25	3.6	7.4	72	12	230	
		32	5	10	24-40 hrs.	24-40 hrs.		100	40	20	20	Initial		8.0	7.7	86	4	200	24 Hr. D.O. - 3.0
												96 hrs.		4.8	7.6	86	10	190	
		18	5	10	46 hrs.			100	60	60	60	Initial		8.0	8.0	96	4	175	
												96 hrs.		4.2	7.6	96	8	155	
	TLm	(Median Tolerance Limit) - Per cent concentration						42	28	21	21								

APPENDIX TABLE A2 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant A	2-16-56	100										Effluent		6.8	3.8	0	100	150	Dark blue
Effluent																			Burnt rubber odor
24-Hr.Composite		56	2	2	1.5hrs	1.5 hrs.	1.5 hrs.	0	0	0	0	Initial		-	-	-	-	-	No turbidity
In Lab												1.5 hrs.		7.0	5.8	20	40	130	
3:00 PM 2-16		32	5	10	3-17hrs.	3-17 hrs.	3-17 hrs.	0	0	0	0	Initial	25	7.2	6.7	50	26	130	
Fish Added												17 hrs.	25	6.4	7.0	48	16	125	
3:00 PM 2-19		18	5	10	3-17hrs.	26 hrs.	26 hrs.	60	0	0	0	Initial		7.4	7.1	68	16	130	
												48 hrs.		5.4	7.6	74	8	130	
		10	5	10				100	100	100	100	Initial		7.6	7.4	94	10	130	
												96 hrs.		4.8	7.7	86	6	130	
	Tlm (Median Tolerance Limit) - Per cent Concentration							32	13.5	13.5	13.5								
Plant A	2-23-56	100										Effluent		9.0	3.3	0	150	145	Dark blue-green
Effluent																			Burnt rubber odor
24-Hr.Composite		56	2	2	15 min.	15 min.	15 min.	0	0	0	0	Initial		-	-	-	-	-	No turbidity
In Lab												15 min.		8.0	3.6	0	92	140	
9:30 AM 2-23		32	5	10	2 hrs.	2 hrs.	2 hrs.	0	0	0	0	Initial	25	7.6	5.8	18	42	135	
Fish Added												2 hrs.	25	7.4	6.1	24	32	135	
11:30 AM 2-23		18	5	10	28-46 hrs	28-46 hrs	28-46 hrs	100	0	0	0	Initial		7.6	6.8	56	22	135	48-Hr.B.O. = 2.2
												46 hrs.		2.2	7.2	60	20	125	
		10	5	10	28-46hrs			100	60	60	60	Initial		7.6	7.2	74	10	130	
												96 hrs.		5.2	7.6	80	4	140	
		5.6	5	10	53-72hrs			100	100	80	80	Initial		7.4	7.4	94	6	130	
												96 hrs.		4.2	7.6	92	4	140	
		Control	5	10				100	100	100	100	Initial		7.4	8.2	98	0	125	
												96 hrs.		5.0	7.7	102	4	130	
	Tlm (Median Tolerance Limit) - Per cent Concentration							56	11	11	11								
Plant A	2-23-56	100										Effluent		8.4	7.4	58	20	330	Slight precipitate when neutralized.
Same as above																			
Neutralized		56	5	10	6-21 hrs	6-21 hrs	6-21 hrs	0	0	0	0	Initial	25	7.8	7.8	80	12	250	Lower concentration not set up.
with lime												21 hrs.	25	6.0	7.4	80	14	240	
		32	5	10	6-21 hrs	6-21 hrs	6-21 hrs	0	0	0	0	Initial		7.6	8.0	92	8	195	No reduction in toxicity indicated from neutralization.
												21 hrs.		4.4	7.5	90	12	190	

APPENDIX TABLE A3 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - BATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant A	3-14-56	100										Effluent		6.8	5.6	24	30	130	Greenish-blue Faint solvent odor Slight turbidity
Effluent																			
24-Hr.Composite		56	2	2	3-15 hrs	3-15 hrs	3-15 hrs	0	0	0	0	Initial		-	-	-	-	-	
In Lab																			
2:00 PM 3-14		32	5	10	3-15 hrs	3-15 hrs	3-15 hrs	0	0	0	0	Initial	23	8.0	7.3	64	12	120	No pH effect
Fish Added												16 hrs.	24	7.4	7.3	66	12	120	
5:00 PM 3-14		18	10	20	3-15 hrs	3-15 hrs	20 hrs	0	0	0	0	Initial		8.4	7.6	74	6	115	
												20 hrs.		7.4	7.8	76	8	125	
		10	10	20	3-15 hrs	24 hrs	44 hrs	50	0	0	0	Initial		8.4	7.8	80	4	115	
												44 hrs.		5.2	7.5	84	6	120	
		5.6	10	20				100	100	100	100	Initial		8.4	8.0	84	4	120	
												96 hrs.		5.0	7.7	86	6	120	
		Control	10	20				100	100	100	100	Initial		8.4	8.0	90	2	115	
												96 hrs.		5.8	7.8	92	6	115	
		TLm (Median Tolerance Limit) - Per cent concentration						10	7.5	7.5	7.5								
Plant A	3-21-56	100										Effluent		6.6	3.0	0	155	185	Bright green Scotish solvent odor Slight turbidity
Effluen																			
24-Hr.Composite		32	2	2	6-18 hrs	6-18 hrs	6-18 hrs.	0	0	0	0	Initial		-	-	-	-	-	No pH effect Black precipitate when water added
In Lab												18 hrs		6.8	6.7	40	14	145	
10:00 AM 3-21		18	5	10	3-15 hrs	23 hrs	24-39 hrs	40	0	0	0	Initial	24	8.2	6.7	62	22	130	
Fish Added												39 hrs	24	0.6	6.9	56	16	140	Low D.O.
5:00 PM 3-21		10	5	10	24-39 hrs	44 hrs	47 hrs	100	0	0	0	Initial		9.0	6.9	72	14	125	
												47 hrs		3.6	7.2	72	14	135	
		5.6	5	10	24-39 hrs			100	80	80	60	Initial		9.0	7.4	82	6	125	48 hr. D.O. - 3.4
												96 hrs.		5.6	7.4	82	8	120	
		Control	5	10				100	100	100	100	Initial		9.0	8.1	90	2	120	
												96 hrs.		5.8	7.3	92	6	120	
		TLm (Median Tolerance Limit) - Per cent concentration						16	7	7	6.2								
Plant A		32	2	2	1 hr	1 hr	1 hr	0	0	0	0	Initial		-	-	-	-	-	
Same as above												1 hr		7.2	6.1	-	-	-	
Shiners		18	2	2	3 hrs	3 hrs	5-18 hrs	0	0	0	0	Initial		8.2	6.7	-	-	-	
(Notropis												18 hrs		4.4	7.2	-	-	-	
atherinoides		10	5	10	5-18 hrs	5-18 hrs	22 hrs	0	0	0	0	Initial	23	8.2	7.1	-	-	-	
used as												22 hrs	24	5.2	7.6				
test fish		5.6	5	10	5-18 hrs	29 hrs		80	20	20	20	Initial		8.2	7.3	-	-	-	24 hr. D.O. - 2.4
												96 hrs.		6.2	7.8	86	4	125	
		Control	5	10				100	100	100	100	Initial		9.0	8.1	-	-	-	
												96 hrs.		6.2	7.6	96	4	120	
		TLm (Median Tolerance Limit) - Per cent concentration						7	<4.5	<4.5	<4.5								

APPENDIX TABLE A4 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant A	6-21-56	100										Effluent		-	7.8	144	10	135	Dark Green Paint Solvent Odor No turbidity
Effluent																			
8-Hr.Composite		32	5	10	5 hrs.	7 hrs.	8 hrs.	0	0	0	0	Initial	23	5.2	8.0	110	6	135	
7 AM - 3 PM 6-21												8 hrs.	23	3.8	8.0	110	6	135	
Fish Added		18	5	10	8 hrs.	8-23 hrs.	8-23 hrs.	0	0	0	0	Initial		6.0	8.1	100	5	135	24 hr. D.O. - 6.0
10 AM 6-22												23 hrs.		0.8	7.4	110	8	130	Low D.O.
		10	5	10	8-23 hrs.	24 hrs.	48 hrs.	40	0	0	0	Initial		6.4	8.1	100	3	135	
												48 hrs.		4.8	7.8	96	4	130	
		5.6	5	10	8-23 hrs.			60	60	60	60	Initial		7.0	8.1	100	3	135	
												96 hrs.		6.0	7.8	100	4	130	
		Control	5	10				100	100	100	100	Initial		7.8	8.2	98	0	135	
												96 hrs.		4.8	7.7	96	4	130	
	Tlm (Median Tolerance Limit) - Per cent Concentration							7.5	6.2	6.2	6.2								
Plant A	6-21-56	18	5	10	5-24 hrs.	5-24 hrs.	5-24 hrs.	0	0	0	0	Initial	23	-	-	-	-	-	Some reduction in toxicity when oxygen maintained
Effluent												24 hrs.	23	5.0	7.8	104	8		
Same as above		10	5	10	28-45 hrs			100	60	60	60	Initial		-	-	-	-	-	130
Oxygenated												96 hrs.		6.8	8.1	104	2		
Fish Added		5.6	5	10	5-28 hrs			80	80	80	80	Initial		-	-	-	-	-	135
11:00 AM 6-23												96 hrs.		5.0	7.8	100	2		
																		135	

APPENDIX TABLE B1 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	P H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plants B & C	1-18-56	100																	
Effluent												Effluent		0	2.6	0	380	740	Dark red orange
24-Hr. Composite		32	10	20	2 hrs.	3 hrs.	3 hrs.	0	0	0	0								Faint solvent oder
In Lab												Initial	24	3.8	4.6	10	90	850	Slight turbidity
2:00 PM 1-18		24	5	10				100	100	100	100	3 hrs.	23	3.6	4.7	-	70	240	Low pH
Fish Added												Initial		5.2	5.9	26	60	200	
5:30 PM 1-18		18	10	20								96 hrs.		2.8	5.7	20	20	200	
								100	100	100	100	Initial		5.2	6.0	30	35	190	
												96 hrs.		5.2	6.8	20	6	180	
		Control	10	20				100	100	100	100	Initial		7.3	8.0	96	2	128	
												96 hrs.		6.8	7.8	100	4	130	
		TLm (Median Tolerance Limit) - Per cent concentration						28	28	28	28								
Plants B & C	1-19-56	100																	
Effluent												Effluent		-	2.4				Dark red
24-Hr. Composite		32	2	2	30 min.	30 min.	45 min.	0	0	0	0								Faint solvent oder
In Lab												Initial	23	-	-	-	-	-	Slight turbidity
3:00 PM 1-19		18	2	2								1 hr.	23	3.4	3.4	-	-	-	
Fish Added								100	100	100	100	Initial		-	-	-	-	-	Low pH
4:30 PM 1-19		10	2	2								96 hrs.		-	-	-	-	-	
								100	100	100	100	Initial		-	-	-	-	-	
												96 hrs.		-	-	-	-	-	
		TLm (Median Tolerance Limit) - Per cent concentration						24	24	24	24								
Plants B & C	1-19-56	75	2	4	6-21 hrs	6-21 hrs	30-44 hrs	50	0	0	0	Initial	23	3.4	7.0	64	20	650	
Effluent												44 hrs.	23	0.2	6.8	60	20	680	Low D.O.
Same as above		56	5	10	6-21 hrs	30-44 hrs		80	20	20	20	Initial		4.6	7.1	72	20	580	Some precipitation
Neutralized												96 hrs.		-	-	-	-	-	when neutralized
With lime		32	5	10				100	100	100	100	Initial		5.8	7.3	80	10	400	with lime
Fish Added 12:30 PM 1-23												96 hrs.		-	-	-	-	-	
		TLm (Median Tolerance Limit) - Per cent concentration						75	46	46	46								
Plants B & C	2-14-56	100																	
Effluent												Effluent		2.0	2.9	0	660	800	Dark red-orange
24-Hr. Composite		32	5	10	30 min.	1 hr.	1 hr.	0	0	0	0								No oder
In Lab												Initial	25	5.6	3.8	0	185	150	Slight turbidity
10:00 AM 2-14		24	10	20	1.5 hrs.	3 hrs.	4 hrs.	0	0	0	0	1 hr.	25	5.4	3.7	0	140	155	Low pH
Fish Added												Initial		5.4	4.9	6	90	150	
4:00 PM 2-14		18	10	20	45 hrs.	72 hrs.		100	70	50	10	4 hrs.		4.8	4.5	10	68	150	
												Initial		6.2	5.8	28	85	150	48 hr.D.O. - 1.4
												96 hrs.		2.0	6.7	20	12	150	Aerated after 48 hrs.
		10	10	20	48 hrs.	72-90 hrs		100	90	60	10	Initial		6.6	6.5	56	40	145	
												96 hrs.		3.0	7.1	56	140		Aerated after 48 hrs.
		Contrl	10	20				100	100	100	100	Initial		7.6	8.1	98	1	125	
												96 hrs.		4.6	7.8	100	6	125	
		TLm (Median Tolerance Limit) - Per cent concentration						21	19	18	< 10								

APPENDIX TABLE B2 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TFS, SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	P H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plants B & C	2-14-56	100										Effluent			7.0				Dark brown
Effluent																			Precipitate formed
Same as above		75	2	2	3 hrs	3 hrs	4 hrs	0	0	0	0	Initial		4.6	7.2	48	12	540	when neutralized
Neutralized												4 hrs.		5.0	7.2	50	4	520	
With lime		56	5	10	26-45 hrs	45-48 hrs	48 hrs	100	0	0	0	Initial	25	5.6	7.6	64	8	430	Aerated after 24 hrs.
Fish Added												48 hrs.	25	4.4	7.3	64	8	445	
11:30 AM 2-15		32	5	10	53 hrs	53-71 hrs	72 hrs	100	100	0	0	Initial		6.0	7.7	84	8	315	Aerated after 24 hrs.
												72 hrs.		4.6	7.5	84	6	310	
		Control	5	10				100	100	100	100	Initial		8.0	8.0	96	2	125	
												96 hrs.		4.2	7.7	100	4	130	
		TLm (Median Tolerance Limit) - Per cent Concentration						65	42	<32	<32								
Plants B & C	2-21-56	100										Effluent		2.0	3.2	0	350	280	Dark orange-red
Effluent																			No odor
24-Hr.Composite		56	2	2	1 hr.	1 hr.	1.5 hrs	0	0	0	0	Initial		-	-	-	-	-	Slight turbidity
In Lab												1.5 hrs		2.6	3.6	0	175	235	Low pH
9:30 AM 2-21		32	5	10	5 hrs	7 hrs	7-21 hrs	0	0	0	0	Initial	25	5.6	5.7	8	85	180	
Fish Added												21 hrs	25	4.4	5.5	6	55	200	
12:00 N 2-21		18	5	10	24 hrs	77-93 hrs		80	80	80	40	Initial		6.4	6.4	34	34	170	
												96 hrs		3.2	7.3	38	8	155	
		10	5	10				100	100	100	100	Initial		7.6	6.8	64	24	140	
												96 hrs		3.8	7.5	66	8	145	
		Control	10	20				100	100	100	100	Initial		8.4	8.1	100	1	130	
												96 hrs		4.2	7.7	102	4	125	
		TLm (Median Tolerance Limit) - Per cent Concentration						22	22	22	16								
Plants B & C	2-27-56																		
Same as above		100										Effluent			7.0				Small amount of
Neutralized																			brown precipitate
With lime		56	5	10	24-42 hrs	43 hrs		100	40	40	40	Initial	25	5.8	7.2	52	20	390	Low 48 hr.D.O. - 0.6
Fish Added												96 hrs	25	6.8	7.7	68	6	385	Aerated after 48 hrs.
3:00 PM 2-21		28	5	10				100	100	100	100	Initial		6.6	7.4	72	8	255	
												96 hrs		1.6	7.4	86	6	265	72 hr.D.O. - 2.4
		Control	10	20				100	100	100	100	Initial		8.4	8.1	100	1	130	
												96 hrs		4.2	7.7	102	4	125	
		TLm (Median Tolerance Limit) - Per cent Concentration						>56	47	47	47								

APPENDIX TABLE B3 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENE HARDNESS ppm.	
Plants B & C	3-13-56	100										Effluent		0	2.9	0	610	480	Dark orange-red Faint burnt rubber odor Slight turbidity
Effluent																			
24-Hr.Composite		32	5	10	15 min	30 min	30 min	0	0	0	0	Initial	23	2.4	3.4	0	200	210	Low pH
In Lab												30 min	23	2.0	3.5	0	190	210	
10:00 AM 3-13		18	10	20	2 hrs	3 hrs	4 hrs	0	0	0	0	Initial		4.4	4.5	4	100	180	Low pH
Fish Added												4 hrs		4.2	4.5	4	105	180	
3:00 PM 3-13		10	10	20	-	-	-	100	100	100	100	Initial	23	5.6	6.1	34	52	160	48 hr.D.O. - 1.2 Oxygen added after 48 hrs.
												96 hrs	24	6.0	7.4	24	6	155	
		5.6	10	20	-	-	-	100	100	100	100	Initial		6.6	6.6	60	28	150	
												96 hrs		3.6	7.4	54	6	140	
		Control	10	20	-	-	-	100	100	100	100	Initial		8.0	8.1	96	1	125	
												96 hrs		4.2	7.7	94	4	120	
		TLm (Median Tolerance Limit) - Per cent Concentration						13.5	13.5	13.5	13.5								
Plants B & C	3-13-56	100										Effluent		-	6.7	44	18	1050	Heavy dark brown precipitate when lime added.
Effluent																			
Same as above		56	2	2	35-47 hrs	35-47 hrs	35-47 hrs	100	0	0	0	Initial	23	-	-	-	-	-	
Neutralized												47 hrs	24	3.8	6.9	70	6	650	
With Lime		32	5	10	48 hrs	-	-	100	80	80	60	Initial		2.8	6.7	54	36	365	Oxygen added after 24 hrs.
Fish Added												96 hrs.		6.0	7.1	54	20	415	
12:00 N 3-19		18	5	10	-	-	-	100	100	100	100	Initial		4.6	7.2	78	18	295	
												96 hrs.		3.6	7.2	84	10	275	
		Control	10	20	-	-	-	100	100	100	100	Initial		7.8	8.3	92	2	115	
												96 hrs.		6.2	7.6	100	6	130	
		TLm (Median Tolerance Limit) - Per cent Concentration						>56	39	39	35								
Plants B & C	3-21-56	100	2	2								Effluent		-	2.5	0	600	300	Dark orange-red. No odor. Slight turbidity.
Effluent																			
24-Hr.Composite		32	5	10	2 hrs	2 hrs	2 hrs	0	0	0	0	Initial	23	-	-	-	-	-	
In Lab												2 hrs.	23	6.0	3.2	0	145	200	Low pH
10:00 AM 3-21		18	5	10	3-15 hrs	3-15 hrs	3-15 hrs	0	0	0	0	Initial		7.2	4.9	6	80	170	
Fish Added												15 hrs.		3.0	5.0	10	50	170	
5:00 PM 3-21		10	5	10	-	-	-	100	100	100	100	Initial	23	8.4	6.3	42	42	150	
												96 hrs.	24	4.2	7.0	40	6	145	
		5.6	5	10	-	-	-	100	100	100	100	Initial		8.8	6.8	60	24	135	
												96 hrs.		5.6	7.3	66	6	135	
		Control	5	10	-	-	-	100	100	100	100	Initial		9.0	8.1	90	2	120	
												96 hrs.		5.8	7.3	92	6	120	
		TLm (Median Tolerance Limit) - Per cent Concentration						13.5	13.5	13.5	13.5								

APPENDIX TABLE 24 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS AND SHINERS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	P H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plants B & C	3-21-56	32	2	2	30 min.	30 min.	30 min.	0	0	0	0	Initial		-	-	-	-	-	
												30 min.		5.6	3.1	-	-	-	Low pH
Effluent												Initial		-	-	-	-	-	
Same as above		18	2	2	2 hrs.	2 hrs.	2 hrs.	0	0	0	0	2 hrs.		6.2	5.0	-	-	-	
Shiners												Initial	23	7.2	6.3	-	-	-	
(Notropis		10	5	10	3-18 hrs.	3-18 hrs.	26 hrs.	20	0	0	0	26 hrs.	24	2.6	6.7	-	-	-	Possible low D.O.
atherinoides)												Initial		7.8	6.8	-	-	-	
Fish Added		5.6	5	10	26 hrs.			100	60	60	60	96 hrs.		3.0	6.9	62	8	135	24 hr.D.O. - 2.8
2:00 PM 3-22												Initial		9.0	8.1	-	-	-	
		Control	5	10				100	100	100	100	96 hrs.		6.2	7.6	96	4	120	
		Tlm (Median Tolerance Limit) - Per cent concentration						8	6.2	6.2	6.2								
												Effluent		-	2.6	0	420	520	Dark orange-red Sweetish odor No turbidity
Plants B & C	6-20-56	100																	
Effluent												Initial		4.6	3.8	0	60	250	
24-hr.Composite		32	5	10		1 hr	1 hr.	0	0	0	0	1 hr.		4.4	3.8	0	60	250	Low pH
In Lab												Initial	23	5.2	6.0	22	25	200	48 hr. D.O. - 2.0
3:00 PM 6-20		18	5	10				100	100	100	100	96 hrs.	23	7.0	6.7	14	6	190	Added oxygen to 18 and 32 after 48 hours
Fish Added												Initial		6.4	6.7	50	15	155	
11:00 AM 6-21		10	5	10	24-46 hrs			100	80	80	80	96 hrs.		5.4	7.0	50	8	165	24 hr. D.O. - 4.4
												Initial		7.0	7.1	68	8	140	
		5.6	5	10				100	100	100	100	96 hrs.		5.4	7.3	78	6	155	
												Initial		7.8	8.1	96	0	135	
		Control	10	20								96 hrs.		4.8	7.7	96	4	130	
		Tlm (Median Tolerance Limit) - Per cent concentration						24	24	24	24								

APPENDIX TABLE C1 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant E	1-24-56	100										Effluent	23	0	1.9	0	770	240	Light orange. Very strong solvent odor (chlorobenzene). No turbidity.
Effluent																			
24-Hr.Composite		32	10	20	15 min.	15 min.	15 min.	0	0	0	0	Initial		6.6	2.6	0	185	160	
In Lab												15 min.		7.2	2.6	0	190	160	Low pH
2:00 PM 1-24		18	10	20	30 min.	30 min.	45 min.	0	0	0	0	Initial		7.6	3.0	0	100	145	Low pH
Fish Added												45 min.		7.6	3.0	0	100	145	
11.00 AM 1-25		10	10	20	2 hrs.	2 hrs.	3 hrs.	0	0	0	0	Initial		8.0	5.1	8	36	140	
												3 hrs.		7.4	5.0	6	44	140	
		5.6	10	20	7 hrs.	30 hrs.	48 hrs.	60	0	0	0	Initial	23	7.8	6.3	42	26	130	
												48 hrs.	23	3.0	6.8	50	10	135	
		3.2	10	20	72-96 hrs	-	-	100	100	100	80	Initial		8.0	6.8	68	20	125	
												96 hrs.		4.4	7.4	78	4	140	
		Control	10	20	-	-	-	100	100	100	100	Initial		8.2	8.2	96	1	125	
												96 hrs.		5.2	7.7	106	4	150	
		TLm (Median Tolerance Limit) - Per cent concentration						6.2	4.2	4.2	4.0								
Plant E	1-24-56	18	2	2	1 hr.	1 hr.	1 hr.	0	0	0	0	Initial		-	-	-	-	-	When neutralized, light brown precipitate forms which settles rapidly. Possible low D.O.
Effluent												1 hr.		7.4	7.9	90	2	325	
Same as above		10	2	2	3-16 hrs	3-16 hrs	16-24 hrs	0	0	0	0	Initial		-	-	-	-	-	
Neutralized												24 hrs.		0.8	7.4	98	12	240	
With lime		5.6	2	2	72-96 hrs	-	-	100	100	100	50	Initial	23	-	-	-	-	-	
Fish Added												96 hrs.	23	4.8	7.8	116	4	220	
4:00 PM 1-25		3.2	2	2	-	-	-	100	100	100	100	Initial		-	-	-	-	-	
												96 hrs.		5.4	7.5	86	4	170	
		TLm (Median Tolerance Limit) - Per cent concentration						7.5	7.5	7.5	5.6								
Plant E	2-15-56	100										Effluent	23	9.4	3.0	0	280	150	Light pink. Slight solvent odor. No turbidity
Effluent																			
24-Hr.Composite		56	2	2	1 hr.	1 hr.	1 hr.	0	0	0	0	Initial		-	-	-	-	-	
In Lab												1 hr.		-	-	-	-	-	
1:30 PM 2-25		32	10	20	4 hrs.	4-16 hrs.	4-16 hrs.	0	0	0	0	Initial		8.2	4.7	2	66	130	Possible low pH
Fish Added												16 hrs.		7.4	5.0	4	36	130	
4:30 PM 2-15		18	10	20	-	-	-	100	100	100	100	Initial	25	8.0	6.1	40	34	130	
												96 hrs.	25	3.8	7.3	44	6	140	
		10	10	20	-	-	-	100	100	100	100	Initial		7.8	6.5	70	22	130	
												96 hrs.		3.6	7.4	72	4	135	
		Control	10	20	-	-	-	100	100	100	100	Initial		8.0	8.0	96	2	125	
												96 hrs.		4.2	7.7	100	4	135	
		TLm (Median Tolerance Limit) - Per cent Concentration						24	24	24	24								

APPENDIX TABLE C2 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA						REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant E	2-17-56	100										Effluent		7.0	2.7	0	480	125	Light dirty orange. Faint solvent (sweet) odor.
Effluent																			
24-Hr.Composite		56	2	2	15 min.	15 min.	15 min.	0	0	0	0	Initial	25	-	-	-	-	-	Slight turbidity.
In Lab												15 min.		-	2.9	-	-	-	Low pH
2:00 PM 2-17		32	2	2	15 min.	15 min.	15 min.	0	0	0	0	Initial		-	-	-	-	-	
Fish Added												15 min.		-	3.3	-	-	-	Low pH
1:45 PM 2-18		18	5	10	30 min.	1 hr.	1 hr.	0	0	0	0	Initial		7.4	4.3	0	60	125	
												1 hr.		7.2	4.4	0	40	125	Low pH
		10	5	10	4 hrs.	4-22 hrs	4-22 hrs	0	0	0	0	Initial		7.4	6.6	40	28	125	
												22 hrs.		6.0	6.9	44	20	125	
		5.6	5	10	28-42 hrs	48-66 hrs	48-66 hrs	100	60	0	0	Initial	25	7.6	7.0	66	20	125	
												66 hrs.	25	5.2	7.2	68	6	125	
		3.2	5	10	-	-	-	100	100	100	100	Initial		7.6	7.3	80	10	125	
												96 hrs.		3.2	7.5	90	6	135	
		Tlm (Median Tolerance Limit) - Per cent Concentration						7.5	6.2	4.2	4.2								
Plant E	2-17-56	100										Effluent	25	6.8	7.4	30	4	650	Heavy light colored precipitate when neutralized.
Effluent																			
Same as above		18	5	10	3 hrs.	4 hrs.	4 hrs.	0	0	0	0	Initial		7.4	7.9	82	4	215	
Neutralized												4 hrs.		7.2	7.8	84	6	220	
With lime		10	5	10	4-22 hrs.	4-22 hrs.	25 hrs.	20	0	0	0	Initial		7.4	8.0	90	4	175	
Fish Added												25 hrs.		4.6	7.6	92	4	180	
1:45 PM 2-18		5.6	5	10	34-42 hrs	48-66 hrs	72 hrs.	100	80	0	0	Initial	25	7.6	8.1	94	2	165	
												72 hrs.	25	4.6	7.5	98	6	165	
		Tlm (Median Tolerance Limit) - Per cent Concentration						8	7	4.2	4.2								
Plant E	2-21-56	100										Effluent		10.0	2.5	0	1020	135	Light tan. Faint solvent odor. Some turbidity.
Effluent																			
24-Hr.Composite		32	2	2	15 min.	15 min.	15 min.	0	0	0	0	Initial	25	-	-	-	-	-	
In Lab												15 min.		8.8	3.2	0	300	130	Low pH
10:00 AM 2-21		18	5	10	30 min.	30 min.	30 min.	0	0	0	0	Initial		8.6	3.9	0	142	130	
Fish Added												15 min.		8.4	3.9	0	130	130	Low pH
1:30 PM 2-21		10	10	20	2 hrs.	3 hrs.	4 hrs.	0	0	0	0	Initial		8.6	5.5	8	58	130	
												4 hrs.		8.0	5.5	10	50	130	
		5.6	10	20	76-92 hrs	-	-	100	100	100	80	Initial	25	8.6	6.6	46	36	130	
												96 hrs.	25	3.4	7.4	50	6	130	
		3.2	10	20	-	-	-	100	100	100	100	Initial		8.4	6.9	68	24	130	
												96 hrs.		4.6	7.6	70	4	130	
		Control	10	20	-	-	-	100	100	100	100	Initial		8.4	8.1	100	1	130	
												96 hrs.		4.2	7.7	102	4	125	
		Tlm (Median Tolerance Limit) - Per cent Concentration						7.5	7.5	7.5	7.0								

APPENDIX TABLE 03 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Plant E	2-21-56	100										Effluent		9.0	7.3	90	8	1150	Large amount of a light flocculent precipitate.
Effluent																			
Same as above		32	5	10	2 hrs.	4 hrs.	6 hrs.	0	0	0	0	Initial		7.4	7.8	76	4	400	
Neutralized												6 hrs.		7.0	7.5	76	4	405	
With lime		18	5	10	24 hrs.	48 hrs.	-	80	40	40	40	Initial	25	8.6	7.6	90	6	310	
Fish Added												96 hrs.	25	5.4	7.8	94	6	290	
3:00 PM 2-21		10	5	10	4-18 hrs.	-	-	80	80	80	80	Initial		8.4	7.7	96	4	220	
												96 hrs.		4.4	7.8	100	4	235	
		TLm (Median Tolerance Limit) - Per cent Concentration						22	16	16	16								
Plant E	3-13-56	100										Effluent		8.8	2.6	0	960	135	Dirty blue grey. Strong solvent odor. Slight turbidity.
Effluent																			
24 hr.Composite		32	2	2	15 min.	15 min.	15 min.	0	0	0	0	Initial		-	-	-	-	-	
In Lab												15 min.		8.0	3.0	0	475	135	Low pH
10:00 AM 3-13		18	5	10	1 hr.	1 hr.	1 hr.	0	0	0	0	Initial		8.2	3.4	0	150	130	
Fish Added												1 hr.		8.2	3.4	0	130	130	Low pH
9:00 PM 3-13		10	10	20	3-17 hrs.	3-17 hrs.	3-17 hrs.	0	0	0	0	Initial		8.2	4.1	0	90	130	
												17 hrs.		7.8	4.0	0	46	125	Low pH
		5.6	10	20	-	-	-	100	100	100	100	Initial	23	8.2	6.1	96	94	125	
												96 hrs.	24	3.4	7.1	96	6	135	
		Control	10	20	-	-	-	100	100	100	100	Initial		8.0	8.1	96	1	125	
												96 hrs.		4.2	7.7	94	4	120	
		TLm (Median Tolerance Limit) - Per cent Concentration						7.5	7.5	7.5	7.5								
Plant E	3-13-56	100										Effluent			9.7	90	0	1130	Dark brown flocculent precipitate which settles rapidly.
Effluent																			
Same as above		18	5	10	8-20 hrs.	-	-	80	80	60	60	Initial	23	7.4	8.3	90	2	305	Toxicity may have been reduced on standing.
Neutralized												96 hrs.	24	4.2	7.5	90	10	325	
With lime		10	5	10	56-68 hrs.	-	-	100	100	80	80	Initial		7.6	8.3	90	2	235	
Fish Added-12:00 Noon 3-19												96 hrs.		4.8	7.6	96	8	235	
		TLm (Median Tolerance Limit) - Per cent Concentration						>18	>18	>18	>18								
Plant E	3-20-56	100										Effluent		4.4	2.3	0	1200	180	Pink. Sweetish solvent odor. No turbidity.
Effluent																			
24-Hr.Composite		32	2	2	30 min.	30 min.	30 min.	0	0	0	0	Initial		-	-	-	-	-	
In Lab												30 min.		6.8	3.0	0	394	135	Low pH
10:00 AM 3-20		18	2	2	6-21 hrs.	6-21 hrs.	6-21 hrs.	0	0	0	0	Initial		-	-	-	-	-	
Fish Added-												21 hrs.		7.4	4.1	0	140	140	Low pH
11:00 AM 3-20		10	2	2	6-21 hrs.	6-21 hrs.	6-21 hrs.	0	0	0	0	Initial		-	-	-	-	-	
												21 hrs.		8.2	4.7	2	44	130	Low pH
		5.6	2	2	48 hrs.	48 hrs.	-	100	50	50	50	Initial	23	-	-	-	-	-	
												96 hrs.	24	6.0	7.0	24	4	130	
		TLm (Median Tolerance Limit) - Per cent Concentration						7.5	5.6	5.6	5.6								

APPENDIX TABLE 5A -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS AND SHINERS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH: FIRST FISH	TIME OF DEATH: HALF OF FISH	TIME OF DEATH: ALL OF FISH	PERCENT SURVIVAL: 24 HOURS	48 HOURS	72 HOURS	96 HOURS	CHEMICAL AND PHYSICAL DATA: TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm.	VERSENATE HARDNESS ppm.	REMARKS
Plant E	3-21-56	100										Effluent	23	10.4	2.5	0	680	185	Pink
Effluent																			Strong solvent odor
24-Hr.Composite		18	2	2	2 hrs.	2 hrs.	3 hrs.	0	0	0	0	Initial		-	-	-	-	-	No turbidity
In Lab												3 hrs.		8.2	4.5	4	85	145	
10:00 AM 3-21		10	5	10	3 hrs	3-15 hrs.	3-15 hrs.	0	0	0	0	Initial		8.6	5.9	20	52	125	Low pH
Fish Added												15 hrs.		7.5	5.9	28	30	125	Heavy pink precipitate
5:00 AM 3-21		5.6	5	10	3-15 hrs.	24 hrs.	24-39 hrs.	20	0	0	0	Initial		9.0	6.7	52	28	125	is all lower concentrations.
												39 hrs.		4.6	6.9	50	12	125	With mortality as
		3.2	5	10	-	-	-	100	100	100	100	Initial	23	9.0	6.9	66	18	125	this pH value, neutralization would
												96 hrs.	24	5.0	7.3	74	8	120	not be expected to
		Control	5	10	-	-	-	100	100	100	100	Initial		9.0	8.1	90	2	120	decrease toxicity.
												96 hrs.			5.8	7.3	92	6	120
		Tlm (Median Tolerance Limit) - Per cent Concentration						3.5	3.2	3.2	3.2								
Plant E	3-21-56	10	2	2	1 hr.	1 hr.	1.5 hrs.	0	0	0	0	Initial		-	-	-	-	-	
Effluent												1.5 hrs.		7.6	5.8	-	-	-	
Same as above		5.6	5	10	2 hrs.	2 hrs.	3 hrs.	0	0	0	0	Initial		-	-	-	-	-	All fish died in con-
Shiners												3 hrs.		8.0	6.5	-	-	-	centrations set up.
(Notropis		3.2	5	10	4 hrs.	4-19 hrs.	4-19 hrs.	0	0	0	0	Initial	23	-	-	-	-	-	Complete survival in
atherinoides)												19 hrs.	24	5.2	7.1	-	-	-	the next lower con-
Fish Added		Control	5	10	-	-	-	100	100	100	100	Initial		-	-	-	-	-	centration (1.68) would
3:00 PM 3-22												96 hrs.		6.2	7.6	96	4	120	give a Tlm value of
		Tlm (Median Tolerance Limit) - Per cent Concentration						< 2.5	< 2.5	< 2.5	< 2.5								2.45
Plant E	6-20-56	100																	
Effluent												Effluent	23	-	2.6	0	245	210	Light dirty orange
24-Hr.Composite		32	5	10	1 hr.	2 hrs.	3 hrs.	0	0	0	0	Initial		7.6	4.8	6	52	150	Solvent odor
In Lab												3 hrs.		7.6	4.8	6	52	150	Slight turbidity
3:00 PM 6-20		18	5	10	7 hrs.	-	-	60	60	60	60	Initial	23	8.0	6.1	36	26	135	Low pH
Fish Added												96 hrs.	23	4.8	7.1	40	8	145	
11:00 AM 6-21		10	5	10	-	-	-	100	100	100	100	Initial		8.0	6.6	62	14	135	
												96 hrs.		4.8	7.3	70	4	135	
		5.6	5	10	72 hrs.	-	-	100	100	80	80	Initial		8.2	6.9	74	10	135	
												96 hrs.			5.8	7.6	82	4	135
		Control	10	20	96 hrs.	-	-	100	100	100	90	Initial		7.8	8.2	98	0	135	
												96 hrs.			4.8	7.7	96	4	130
		Tlm (Median Tolerance Limit) - Per cent Concentration						20	20	20	20								

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APPENDIX TABLE B1 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH: FIRST FISH	TIME OF DEATH: HALF OF FISH	TIME OF DEATH: ALL OF FISH	PERCENT SURVIVAL: 24 HOURS	48 HOURS	72 HOURS	96 HOURS	CHEMICAL AND PHYSICAL DATA: TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm.	VERSENATE HARDNESS ppm.	REMARKS
Mixed A, B & C	3-21-56	100										Effluent	23	4.0	2.6	0	320	230	Dark brownish purple
and D Effluents																			Sweetish solvent odor
Plant A 3-14		32	2	2	15 min	15 min	15 min	0	0	0	0	Initial		-	-	-	-	-	Some turbidity
B & C 3-13												15 min		6.2	4.2	0	75	155	Low pH
D 3-13		18	5	10	2 hrs	2-16 hrs	2-16 hrs	0	0	0	0	Initial		6.2	6.0	30	44	135	
Mixed 3:00 PM 3-20												16 hrs		5.6	6.3	22	20	130	With mortality at
[illegible]		10	5	10	2-16 hrs	2-16 hrs	-	40	40	40	40	Initial	23	7.6	6.5	58	26	130	pH 6 to 6.5, neu-
volume released												96 hrs	24	5.4	7.5	54	6	135	tralization would
Fish Added		5.6	5	10	-	-	-	100	100	100	100	Initial		8.0	6.9	74	14	125	not be expected to
5:00 PM 3-16												96 hrs		5.0	7.6	70	6	135	greatly reduce
		Control	5	10	-	-	-	100	100	100	100	Initial		8.6	8.2	90	1	115	toxicity.
												96 hrs			5.8	7.7	96	4	125
		Tlm (Median Tolerance Limit) - Per cent Concentration						9	9	9	9								
Mixed A, B & C	3-21-56	100										Effluent	24	4.4	2.6	0	400	280	Dark brown
and D Effluents																			Solvent odor
All Collected 3-2		32	2	2	1 hr	1 hr	1 hr	0	0	0	0	Initial		-	-	-	-	-	Small amount of
Mixed 3:00PM 3-2												1 hr		6.8	4.3	0	70	180	precipitate
In proportion to		18	5	10	2-14 hrs	15 hrs	20 hrs	0	0	0	0	Initial		7.2	6.2	36	44	150	Low pH
volume released												20 hrs		6.4	6.0	30	30	150	With complete mor-
Fish Added		10	5	10	-	-	-	100	100	100	100	Initial	24	8.2	6.7	60	26	135	tality at pH 6.2,
6:30 PM 3-21												96 hrs	24	4.0	7.2	56	8	140	neutralization would
		5.6	5	10	-	-	-	100	100	100	100	Initial		8.4	6.9	76	16	130	not be expected
												96 hrs		5.0	7.4	74	6	130	to reduce toxicity.
		Control	5	10	-	-	-	100	100	100	100	Initial		9.0	8.1	90	2	120	
												96 hrs			5.8	7.3	92	6	120
		Tlm (Median Tolerance Limit) - Per cent Concentration						13.5	13.5	13.5	13.5								
Mixed A, B & C	6-21-56	100										Effluent	23	-	5.5	24	100	325	Dark red-orange
and D Effluents																			Sweetish solvent odor
A - 6-21		32	5	10	2-20 hrs	2-42hrs	2-20 hrs	0	0	0	0	Initial		5.0	6.5	62	30	185	
B & C, D 6-20												20 hrs		1.2	7.0	64	16	190	Possible low D.O.
Mixed 1:00PM 6-23		18	5	10	2-20 hrs	2-20 hrs	24-42 hrs	20	0	0	0	Initial		6.0	6.8	74	16	170	with mortality at
in proportion												42 hrs			7.0	78	8	170	pH 6.8, neutralization
to volume released		10	5	10	2-20 hrs	72-90 hrs	-	80	60	60	40	Initial	23	6.8	7.1	82	8	150	would not be expected
Fish Added												96 hrs	23	5.2	7.6	90	4	135	to reduce toxicity.
2:00 PM 6-23		5.6	5	10	42 hrs	-	-	100	80	80	80	Initial		7.4	7.3	92	6	135	
												96 hrs		5.2	7.6	88	4	130	
		Control	10	20	96 hrs	-	-	100	100	100	90	Initial		7.8	8.1	94	0	135	
												96 hrs			4.8	7.6	96	4	130
		Tlm (Median Tolerance Limit) - Per cent Concentration						13.5	11	11	8.7								

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APPENDIX TABLE B-1 -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH: FIRST FISH	TIME OF DEATH: HALF OF FISH	TIME OF DEATH: ALL OF FISH	PERCENT SURVIVAL: 24 HOURS	48 HOURS	72 HOURS	96 HOURS	CHEMICAL AND PHYSICAL DATA: TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm.	VERSENATE HARDNESS ppm.	REMARKS
River Intake	1-18-56	100	10	20	40 hrs.	-	-	100	90	90	70	Initial	20	8.0	6.7	72	30	216	Brown.
6-Hr.Composite												96 hrs.	23	5.4	7.3	68	4	225	Faint oil and sewage
In Lab		56	5	10	-	-	-	100	100	100	80	Initial	23	7.8	6.9	80	16	186	odor.
2:00 PM 1-18												96 hrs	23	6.2	7.6	80	4	180	Slight turbidity.
Fish Added		Control	10	20	-	-	-	100	100	100	100	Initial		7.3	8.0	96	2	186	
5:30 PM 1-18												96 hrs.			6.8	7.8	100	4	130
		No Tlm Value - 100% survival in 96 hrs.																	
River Intake	2-14-56	100	10	20	-	-	-	100	100	100	100	Initial	18	11.0	7.3	80	6	135	Light greyish tan.
6-Hr.Composite												96 Hrs.	25	4.0	7.6	80	6	135	Faint sewage odor.
In Lab		56	10	20	-	-	-	100	100	100	100	Initial	23	9.2	7.5	92	4	135	Slight turbidity.
10:00 AM 2-14												96 Hr..	25	5.0	7.7	90	6	130	
Fish Added		Control	10	20	-	-	-	100	100	100	100	Initial	25	7.6	8.1	98	1	125	
5:00 PM 2-14												96 Hrs.	25		4.6	7.8	100	4	130
		No Tlm Value - 100% survival in 96 hrs																	
River Intake	2-16-56	100	10	20	-	-	-	100	100	100	100	Initial	25	7.6	7.6	64	4	110	Light greyish tan.
6-Hr.Composite												96 Hrs.	25		2.6	7.6	70	6	115	Faint sewage odor.
In Lab																			Slight turbidity.
2:00 PM 2-15																			
Fish Added																			
3:00 PM 2-15																			
		No Tlm Value - 100% survival in 96 hrs.																	
River Intake	2-21-56	100	10	20	5-24 hrs.	-	-	90	90	90	90	Initial	25	9.0	7.5	66	6	125	Light greyish tan.
6-Hr.Composite												96 hrs.			4.6	7.8	64	6	125	Gasoline and sewage
In Lab		56	10	20				100	100	100	100	Initial		8.4	7.7	80	6	125	odor.
11:30 AM 2-21												96 hrs		5.4	7.5	86	4	125	Slight turbidity.
Fish Added		Control	10	20				100	100	100	100	Initial		8.4	8.1	100	1	130	
3:00 AM 2-22												96 hrs			5.0	7.6	102	4	130
		No Tlm Value - 90% survival in 96 hrs.																	
River Intake	3-13-56	100	10	20	80 hrs.	-	-	100	100	100	90	Initial	24	10.0	7.5	72	4	115	Light greyish tan.
6-Hr.Composite												96 hrs.			5.0	7.4	72	6	110	Faint gasoline odor.
In Lab		56	5	10				100	100	100	100	Initial		9.0	7.7	80	2	115	Slight turbidity.
2:00 PM 3-13												96 hrs.		5.8	7.6	82	6	120	
Fish Added		Control	10	20				100	100	100	100	Initial		8.0	8.1	90	1	125	
3:00 AM 3-14												96 hrs.			5.4	8.0	98	4	125
		No Tlm Value - 90% survival in 96 hrs.																	

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APPENDIX TABLE XX -BIOASSAY DATA

NATIONAL ANILINE DIVISION, ALLIED CHEMICAL AND DYE CORPORATION
BUFFALO, NEW YORK

TEST FISH - FATHEAD MINNOWS AND SHINERS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
River Intake	3-21-56	100	5	10	-	-	-	100	100	100	100	Initial	23	10.8	7.2	86	12	155	Grayish tan
8-Hr.Composite												96 hrs.		5.2	7.4	90	6	155	Faint gasoline odor
In Lab		56	5	10	-	-	-	100	100	100	100	Initial		9.4	7.5	88	6	140	Slight turbidity
10:00 AM												96 hrs.		5.6	7.5	92	8	135	
Fish Added		Control	5	10	-	-	-	100	100	100	100	Initial		9.0	8.1	90	2	120	
7:30 PM 3-21												96 hrs.		5.8	7.3	92	6	120	
		No TLm Value		100% Survival in 96 hrs.															
River Intake	3-21-56	100	5	10	72 hrs	-	-	100	100	80	80	Initial	23	9.6	7.1	88	10	160	Same as above
Same as above												96 hrs.		4.4	7.6	96	6	155	
Shiners		56	5	10				100	100	100	100	Initial		9.0	7.4	90	6	150	
(Notropis												96 hrs.		4.8	7.7	98	4	130	
atherinoides		32	5	10				100	100	100	100	Initial		9.0	7.7	90	4	135	
Fish Added												96 hrs.		5.0	7.7	104	4	130	
10:00 AM 3-22		Control	5	10				100	100	100	100	Initial		9.0	8.1	90	2	120	
												96 hrs.		6.2	7.6	96	4	120	
		No TLm Value		80% Survival in 96 hrs.															
River Intake	6-21-56	100	5	10	2 hrs	4 hrs.	4 hrs.	0	0	0	0	Initial	23	0.4	6.8	72	30	250	Dark Brown
8-Hr.Composite												4 hrs.		0.0	6.5	62	35	205	Faint solvent odor
In Lab		56	5	10	29-48 hrs			100	80	80	80	Initial		4.4	6.8	76	12	200	Low D.O.
7-3 PM 6-21												96 hrs		4.6	7.3	78	6	185	
Fish Added		32	5	10	29-48 hrs			100	80	80	80	Initial		6.0	7.1	80	9	170	
11:00 AM 6-22												96 hrs.		5.4	7.7	84	4	160	
		Control	10	20	96 hrs			100	100	100	90	Initial		7.8	8.2	94	0	135	
												96 hrs.		4.8	7.7	96	4	130	
		TLm (Median Tolerance Limit) - Per cent Concentration						75	70	70	70								
Same as above	6-21-56	100	5	10	3 hrs	4 hrs	29-48 hrs	20	0	0	0	Initial	23	4.4	-	-	-	-	
Oxygenated												48 hrs.		5.6	7.4	94	8	225	Same as above.
		56	5	10	7-22 hrs			80	80	80	80	Initial		-	-	-	-	-	No reduction in
												96 hrs.		7.6	7.8	80	4	175	toxicity when
		32	5	10	7-22 hrs			80	80	80	80	Initial		-	-	-	-	-	oxygenated.
												96 hrs.		7.0	7.9	84	4	160	